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Comparative Trials of Antimonial Drugs in Urinary Schistosomiasis

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Chemotherapeutic trials in urinary schistosomiasis are described and discussed. Their design and conduct were based on recommended statistical techniques, now generally accepted as the most appropriate approach to the assessment of antischistosomal drugs.

Randomization produced comparable host groups in whom multiple parasitic infection and radiological urinary tract damage were common. Treatment was with one of three antimonial compounds given at equivalent metallic dosage daily.

Antimony sodium tartrate (AST) and antimony dimercaptosuccinate (TWSb) were equally efficient curatively but both produced many side-effects. Sodium antimonylgluconate (TSAG) was four-fifths as effective but tolerance was superior. Estimations of urinary antimony excretion showed that tissue retention of the metal was related to cure-rates and side-effects. It was concluded that none of the drugs were suitable for mass chemotherapy.

More new non-toxic schistosomicides are urgently needed and for their assessment, the setting-up of multicentre trials, following international agreement on technical methods, is suggested.

INTRODUCTION

Schistosomiasis remains one of the major public health problems of the tropics, and conservative estimates place the number of infected individuals at 150 million (World Health Organization, 1965). It is surprising that advances in the chemotherapy of schistosomiasis have not paralleled the progress made in the treatment of other communicable diseases. Despite intensive research, there were until recently, virtually only two groups of useful drugs—the antimonials and the thioxanthones. Neither can be used with complete safety and both produce side-effects which often lead to a high default rate.

Many workers felt that a standardized approach to chemotherapy should be adopted, and recommendations from the various expert committees of the World Health Organization during the past 15 years were crystallized by the WHO Scientific Group on Research on Bilharziasis (Chemotherapy) in 1959. Modifications, from research bodies and individuals continued, and culminated in the WHO-sponsored protocol for trials of antischistosomal drugs (WHO Scientific Group on Research in Bilharziasis (Chemotherapy) 1966). This comprehensive document proposed the establishment of centres in endemic and non-endemic areas, outlined procedural methods, modes of assessment of chemotherapeutic action of drugs, and suggested future lines of research. Meanwhile, the World Health Organization, the Medical Research Council of Great Britain, and the Government of Tanganyika (now the United Republic of Tanzania) had agreed to establish a Bilharziasis Chemotherapy Centre in Tanzania. In 1963 the centre was built in the grounds of a 415-bed government hospital at Tanga, a port and centre of the sisal industry, some 100 miles (160 km) north of the capital, Dar es Salaam.

The surrounding area is primarily low-lying coastal plain. From November to May temperatures frequently rise above 90°F (32°C) with night temperatures above 75°F (24°C), while from June to October, afternoon temperatures of 70°F–80°F (21°C–27°C) are usual, with night temperatures some 10 deg F to 15 deg F lower. Monsoon rain occurs in April and May (the long rains), and intermittently from October to December (the short rains). The total rainfall ranges from 35 to 70 inches (about 890 mm to 1.5 m) per year. The coastal plain drains the eastern Usambara hills through three main river

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systems, while near the coast there are numerous natural depressions holding water for the greater part of the year. Population density is about 80 persons per square mile (50 per km^2).

Schistosoma haematobium infection is hyperendemic in Tanga province and transmission is widespread; *Bulinus (Physopsis) globosus* is the chief intermediate host and other transmitting species are *B. (P.) nastus* and *B. (P.) africanus ovoideus*. Seasonal fluctuations in population numbers of vector snails occur with breeding peaks following the rains but transmission probably occurs all the year round (Blair, 1956; Maclean, Webbe & Msangi, 1958; Webbe & Msangi, 1958; Webbe, 1959).

S. mansoni transmission does not occur in the area and the occasional cases found were considered to have acquired their infection elsewhere. All subsequent remarks relate to urinary schistosomiasis caused by *S. haematobium*.

The initial work of the centre was concerned with the collection of base-line data, and with the methodology of trials of anti-schistosomal drugs. Three antimonial preparations, sodium antimonylgluconate (TSAG), antimony dimercaptosuccinate (TWSb), and antimony sodium tartrate (AST), were selected for comparison. All were in widespread use, all were effective, but there was little published information from Tanzania on their comparative efficiency.

MATERIAL

The patients treated were adult male labourers from sisal estates. Some 14 000 men are employed in hand-cutting the plants, weeding and processing the fibre. Many come from neighbouring countries and the result is a spectrum of the tribes and diseases of east and central Africa.

METHODS

Trial procedures

A doctor and a technician visited estates, and hospital treatment was offered to any labourer with schistosomiasis. All participation was voluntary.

Race, sex, socio-economic class, species of infection, activity and physical condition were broadly similar in all patients. The chances of preinfection or reinfection were alike as no molluscicides were employed in the region.

No attempt was made to select heavy egg-passers. To ensure the emergence of a representative picture of the results of treatment, admission was from a waiting-list. Randomly selected patients were placed in one of three treatment groups, termed A, B and C. In group A there were 60 patients who were treated with TSAG; group B consisted of 50 patients treated with TWSb; and 50 patients who were treated with AST constituted group C.

On admission to hospital, routine investigations included detailed examination, quantitative estimation of egg-output over timed periods on 2 successive days before treatment, haematological examination, parasitological screening of stool, thick blood films and night blood films for associated protozoal and helminthic infections, electrocardiography, chest X-ray, intravenous pyelography and blood urea estimation. During the treatment period, daily quantitative egg counts on timed urine specimens were performed.

Patients were followed up for 5 successive days at 4, 8 and 12 weeks after treatment. The results of the first 1000 examinations showed that all positive urines were detected during the first 3 consecutive days, and in fact, almost always in the first 2 days. The accuracy of the method of urine examination was confirmed and its efficacy over a 3-day follow-up regime demonstrated. It will be shown that the chance of missing even 1 egg was reduced to negligible proportions. The progress of as many patients as possible was followed for 3 successive days at 6, 9 and 12 months after treatment in an attempt to discover their eventual parasitological fate.

Urine examination

All urines were examined immediately on arrival at the central laboratory and were classified as casual or timed, the former being collected during screening in the field, the latter over a timed period in hospital. Casual specimens were collected at some time between 12 noon and 3 p.m. Timed specimens consisted of all urine passed between 10 a.m. and 2 p.m.—the time of maximum egg-output (Stimmel & Scott, 1956). The volume was measured and the presence or absence of visible blood was noted. A simple procedure for the quantitative examination of urine was evolved and preferred to that of Barlow (1931), or the more elaborate methods of Bell (1962) and Bradley (1962).

The microscopic detection of eggs of *S. haematobium* in the urine is not difficult but in light infections eggs may be missed and hatching tests should be performed. Hatching is regarded as the

most useful test of cure (WHO Expert Committee on Bilharziasis, 1953). Common methods of egg-counting in current use involve either centrifugation or sedimentation of a urine specimen, with a count of the eggs contained in an aliquot of concentrate from a known volume, or the filtration–ninhydrin-staining technique (Bell, 1962; Bradley, 1962). In none of these techniques is hatching utilized, although it is the most sensitive method of viable egg detection. These considerations suggested that the problem of quantifying the hatching procedure should be undertaken. If the total miracidial and dead-egg content of a specimen could be determined, plus the ratio of viable eggs to dead eggs, it could be useful in the early detection of drug action. The method evolved to quantify hatching has been in effective use for 4 years.

Procedure for examination of urine

(1) The urine in the original container, a robust flat-bottomed jar of 1- or 2-pint (ca ½–1 litre) capacity, was well mixed and a random sample of 10 ml was withdrawn by Pasteur pipette from different levels in the container.

(2) The 10 ml-specimen of urine was placed in a graduated conical-ended centrifuge tube and centrifuged at 2000 rev/min for 3 min.

(3) 9 ml of supernatant were withdrawn immediately by Venturi pump and discarded.

(4) Freshly boiled and cooled water was used to dilute the remaining 1 ml of concentrate to 5 ml, thorough mixing and the breaking up of blood-clot and debris being ensured by repeated inversion.

(5) The tubes were exposed to artificial light at room temperature (72°F, 22°C) for 30 min; maximum hatching of miracidia occurred within the first 20 min.

(6) 2 ml of absolute alcohol (methyl alcohol is equally efficient and cheaper) and 7 drops of aqueous eosin were added to each tube which was inverted at least six times to ensure penetration of the stain into any compressed deposit. The hatched miracidia were simultaneously killed, fixed and stained.

(7) The tubes were again centrifuged at 2000 rev/min for 3 min, after which the supernatant was withdrawn leaving 0.1 ml.

(8) A 40-mm by 24-mm cover-slip preparation was made and the miracidia and unhatched egg content of the 0.1 ml of concentrate, which represented the content of the original 10-ml random sample, was counted under the 16-mm objective lens.

(9) After treatment, when minimal egg-content was expected, follow-up urines were allowed to sediment in a conical glass container for 30 min, after which 10 ml from the bottom of the container were withdrawn and subjected to the same process. Sedimentation ensured recovery of all eggs in a specimen.

Comment

The method was simple and involved the use of only one graduated centrifuge tube and a Pasteur pipette, thus dispensing with frequent volume changes. It gave an estimate of the passage, and ratio, of live miracidia and dead eggs. Hatched, stained miracidia were easily recognized. Others, killed on the point of emergence from the egg shell, were counted as viable. It was important to distinguish between two groups of dead eggs. Some unhatched eggs contained a formed miracidium and were termed "recently dead" although it was accepted that a few may have been immature. Some eggs were misshapen and discoloured, or contained a deformed miracidium or a collection of globules, and were termed "old", implying that they had been present in the body for a long time. All the deposit on the slide was examined but no note was taken of empty egg shells. Red cells, pus cells and epithelial cells took up stain but background colour was rarely conspicuous enough to interfere with fairly rapid counting. If a specimen was counted immediately after processing, viable miracidia had already stained, but dead ova, encased in their shells, had not taken up stain entirely. Left overnight, the stain penetrated dead eggs in varying amounts. In practice, there was little difficulty in distinguishing between the different categories.

In any method of counting eggs involving light-microscopy there are occasions when heavy crystalluria or the presence of other urinary debris obscures eggs and renders counting difficult. This may be overcome by the dilution of the final deposit to an arbitrary level and the counting of one or more aliquots of the dilution, with calculations to assess the number of eggs present in the original volume. This is practicable in moderate or high counts, but during follow-up, when few or no eggs are expected, there may be no alternative to multiple counts on a debris-ridden suspension.

All random samples were taken in hospital from the total volume of urine passed between 10 a.m. and 2 p.m. Random samples from casual specimens were from those urines taken in the field at some time between 12 noon and 3 p.m.

Experiments to determine the efficiency of the technique

It was thought that at points (3) and (7) during the examination of urine, loss of miracidia or eggs might occur. The following tests were conducted and the results expressed as numbers of miracidia or eggs or both recovered at different stages of the method, are shown in Table 1.

(1) At point (3), the 9 ml of supernatant were centrifuged at 2000 rev/min for 3 min, the upper 8 ml were withdrawn and discarded, and the remaining 1 ml was subjected to hatching, killing, staining and counting. Table 1 shows that, on two occasions only, one miracidium was isolated.

(2) At point (7), the contents of the tube consisted of 5 ml of hatching fluid, 2 ml of absolute alcohol, and eosin. The stained supernatant was withdrawn to 0.2 ml and centrifuged for 3 min at 2000 rev/min. The upper 6.6 ml of this supernatant were then withdrawn and the remaining 0.2 ml counted. This showed that centrifugal speed was sufficient to ensure the passage of miracidia and eggs through the supernatant to the final concentrate. Table 1 shows that the recovery was nil.

(3) Lastly, at point (7), a final volume of 0.2 ml was counted. The top 0.1 ml and the bottom 0.1 ml of concentrate were examined separately. This demonstrated that, in fact, all miracidia and eggs were carried down into the final 0.1 ml (see Table 1).

These results indicated that, for practical purposes, all the miracidia and eggs in a 10-ml specimen of urine were carried down into the last 0.1 ml by the method adopted.

In a condition where hundreds, and in some cases thousands, of eggs were contained in 10 ml of urine, the aim of counting literally every egg may not be attained, but the minor discrepancies of the technique did not render it invalid, provided it was applied to all urines in comparable trials. Errors in the assessment of both pretreatment and follow-up urines were additionally minimized by examination on consecutive days. A further precaution adopted was that every patient, at all times, was provided with his own individually marked urine container, conical glass sedimentation jar, a pipette and a hatching tube, thus avoiding a false count through " carry over " of miracidia or eggs.

TABLE 1

RECOVERY AND LOSS OF MIRACIDIA AND EGGS FROM URINE AT VARIOUS STAGES OF A HATCHING TECHNIQUE

Patient	Count [a] in original 10 ml by stated method	Experiment 1: count [a] on supernatant	Experiment 2: count [a] on stained supernatant	Experiment 3: count [a] on upper 0.1 ml of final concentrate
1	14/3	0/0	0/0	0/0
2	59/20	0/0	0/0	0/0
3	42/24	0/0	0/0	0/0
4	1 142/75	1/0	0/0	0/0
5	112/25	0/0	0/0	0/0
6	1 194/334	0/0	0/0	0/0
7	2 240/235	0/0	0/0	0/0
8	117/62	0/0	0/0	—
9	0/0	0/0	0/0	—
10	3/2	0/0	0/0	—
11	51/16	1/0	0/0	—
12	2/0	0/0	0/0	—
13	26/4	0/0	0/0	—

[a] Number of hatched miracidia in 10 ml of urine/number of dead eggs present in 10 ml of urine.

Probably the greatest source of error associated with the method is the loss of miracidia or eggs as a result of the adherence of small droplets of fluid to the walls of the tube or to the side of the pipette when the final 0.1 ml is removed for counting. An experiment was devised to assess the extent of this loss, and was combined with a preliminary assessment of the effect of pH on the hatching of eggs.

From M/15 solutions of di-sodium hydrogen orthophosphate and potassium di-hydrogen orthophosphate, buffer solutions at 12 different pH values were prepared ranging in steps from pH 5.288 to pH 8.043. About 200 ml of an egg-containing urine were thoroughly mixed to ensure a homogeneous egg distribution. Random 10-ml samples from this urine were withdrawn by Pasteur pipettes, placed in 12 graduated centrifuge tubes and processed as far as point (4) in the method of urine examination. To the 1-ml concentrate in each of the 12 tubes were added 10 ml of one of the dif-

ferent pH solutions. The tubes were then hatched as in point (5) of the method and the miracidia killed, stained and counted. The bottom of the tubes were washed, using each individual Pasteur pipette, and the washings were counted carefully. A thirteenth tube was hatched by addition of freshly boiled water which had been allowed to cool, as in the normal method. The results are shown in Table 2.

To assess the effects of different pH values on hatching, only miracidial counts were considered. Thus the numerator of the count of washings in Table 2 (column *b*) was added to the initial miracidial count (column *a*) to give the total miracidial recovery (column *e*). Although at first sight it might be thought that hatching was slightly better at lower pH values, no trend can in fact be established from this small series. Applying the simple formula applicable to upward or downward trends,

$$K = [n-2(S+1)]/[(n+1)/3]^{\frac{1}{2}}$$

where n is the number of samples and S is the smaller number of signs (Wallis & Roberts, 1962), the standard normal variable $K=0.463$ giving a two-tail probability of 0.646. The different pH values failed to influence hatching significantly. This is hardly surprising considering the widely varying conditions encountered by the parasite in nature and its continued survival.

Theoretically, eggs in a well-mixed urine should follow the Poisson distribution. Applied as a dispersion test χ^2 was 17.993 with 12 degrees of freedom, a non-significant finding, $0.2 > P > 0.1$, indicating that the observed data in Table 2 were compatible with the Poisson distribution. It should be noted that high egg counts may deviate from the Poisson law, probably due to the tendency of eggs to cluster when their density is high (Uemura, unpublished data, 1964). This is less likely to occur with miracidia which are actively motile organisms not surrounded by clusters of red cells, white cells, or debris, as eggs may be, and is a further reason for attempts to quantify hatching procedures.

Further points to be noted from Table 2 are that dead-egg excretion was a fairly constant

TABLE 2

INFLUENCE OF DIFFERENT pH VALUES OF THE HATCHING FLUID ON THE RECOVERY OF MIRACIDIA AND EGGS BY A HATCHING TECHNIQUE

Tube	pH of added diluent	(a) Miracidial count	(b) Count [a] of washings	(c) Recently dead eggs	(d) Long-dead eggs	(e) Total miracidial recovery [b]	Total miracidial and egg recovery [c]
1	5.288	64	1/1	5	3	65	74
2	5.589	67	1/0	3	7	68	78
3	5.906	77	0/1	8	6	77	92
4	6.239	75	7/3	1	3	82	89
5	6.468	62	1/0	10	5	63	78
6	6.643	58	3/0	1	6	61	68
7	6.813	65	2/0	6	4	67	77
8	6.979	54	1/0	9	3	55	67
9	7.168	55	1/1	2	2	56	61
10	7.381	48	4/0	5	3	52	60
11	7.731	56	1/0	0	4	57	61
12	8.043	64	0/0	3	1	64	68
13	Boiled water control	57	1/0	4	2	58	64

[a] Number of hatched miracidia in 10ml of urine/number of dead eggs in 10 ml of urine.
[b] Sum of column (a) and numerator of column (b).
[c] Sum of column (a), (b), (c) and (d).

9%–10% of total egg recovery, and that miracidia and eggs lost in the droplets adhering to the tube was 2%–3%. The loss can be reduced by the use of a fine pipette for the transfer of fluid from the tube to the slide, by washing the tube and pipette and counting the washings. In follow-up work this loss of 2%–3% could be important as very small numbers of viable eggs were expected and could have been missed. This potential danger was eliminated by the examination of three consecutive daily specimens of urine at follow-up. Uemura (unpublished data, 1964) showed that the chances of missing an egg-positive case could be reduced to a minimum if the sample was taken from a specimen in which the distribution of eggs was made homogeneous, and he tabulated the probability of

TABLE 3

COMPARISON OF PRETREATMENT VARIABLES IN THREE GROUPS OF PATIENTS WITH URINARY SCHISTOSOMIASIS

Observation	Treatment group, treatment and number of patients			Comments
	Group A; TSAG (60)	Group B; TWSb (50)	Group C; AST (50)	
Patients from Tanzania	33/60 (55%)	31/50 (62%)	31/50 (62%)	All χ^2 calculated with Yates' correction. $\chi^2 = 0.747$ with 2 DF [a] $0.7 > P > 0.5$ (NS) [b]
Patients from neighbouring countries	27/60 (45%)	19/50 (38%)	19/50 (38%)	
Less than 5 years' history of haematuria	36/60 (60%)	36/50 (72%)	30/50 (60%)	$\chi^2 = 2.142$ with 2 DF [a] $0.5 > P > 0.3$ (NS) [b]
More than 5 years' history of haematuria	24/60 (40%)	14/50 (28%)	20/50 (40%)	
Specific treatment received	34/60 (57%)	22/50 (44%)	19/50 (38%)	$\chi^2 = 4.058$ with 2 DF [a] $0.2 > P > 0.1$ (NS) [b]
No specific treatment received	26/60 (43%)	28/50 (56%)	31/50 (62%)	
Mean body-weight of group (kg ± SD) [c]	57.6 ± 7.2	59.3 ± 5.7	58.8 ± 7.9	Variances homogenous by Bartlett's test. $\chi^2 = 5.09$ with 2 DF [a] $0.1 > P > 0.05$ (NS) [b]. Means not significantly different. Analysis of variance: $F = 1.282$ for 2 and 157 DF [a] (NS) [b]
Number with enlarged liver or spleen or both	21/60 (35%)	17/50 (34%)	24/50 (48%)	$\chi^2 = 2.633$ with 2 DF [a]; $0.3 > P > 0.2$ (NS) [b]
Number negative	39/60 (65%)	33/50 (66%)	26/50 (52%)	
Number infected with *S. haematobium* only	14/60 (23%)	8/50 (16%)	8/50 (16%)	$\chi^2 = 1.324$ with 2 DF [a]; $0.7 > P > 0.5$ (NS) [b]
Number infected with multiple parasites	46/60 (77%)	42/50 (84%)	42/50 (84%)	
Mean percentage of eosinophilia	15.1	15.5	12.3	
Mean haemoglobin percentage	90.8	89.4	93.6	
Number of pretreatment egg-counts containing stated number of eggs per 10 ml of urine. Each count is from 1 field and 2 timed specimens	1–199 eggs/10 ml, 105/180 (58%);	100/150 (67%)	100/150 (67%)	$\chi^2 = 5.35$ with 4 DF [a]; $0.3 > P > 0.2$ (NS) [a] Proportions of egg-counts similar in the three treatment groups. Analysis of variance of individual counts: $F = 1.249$ for 8 and 471 DF [a] (NS) [b]
	200-999 eggs/10 ml, 55/180 (31%);	41/150 (27%)	41/150 (27%)	
	1000 + eggs/10 ml, 20/180 (11%)	9/150 (6%)	9/150 (6%)	

[a] Degrees of freedom. [b] Not significant. [c] Standard deviation.

detection of eggs corresponding to the expected number of eggs per count when eggs were distributed at random. The probability of detection of only 1 egg on 1 count is 0.63, of only 1 egg on 2 counts is 0.86, and of only 1 egg in 3 counts is 0.95. The probabilities of detection of 2 eggs in 1, 2 and 3 counts are 0.86, 0.98 and 0.99, respectively. As follow-up urines were sedimented to ensure the passage of all eggs into the bottom 10 ml examined, and as 3 counts during follow-up was the standard procedure, the satisfactory detection of as few as 1 or 2 eggs per specimen was attained. The use of a mucilage-tragacanth mixture might be expected to eliminate loss *via* the droplets in the tube and pipette and this possibility is being investigated.

There are numerous sources of variability in egg-counting, inherent in technical methods and observer error. Every method in use is only as reliable as the performing technician, and every method is capable of giving grossly inaccurate results if used improperly or carelessly. Egg-counts are useful in providing a broad picture of group egg-output in prevalence or incidence surveys, and during treatment trials, provided that a critical approach is adopted both to technique and results.

It is not claimed that the method is capable of detecting every egg in a specimen, especially when counts may be as high as 5000 eggs per 10 ml. It was considered to be a practical compromise in dealing with the problem of egg-counting, and accurate enough for use in a laboratory dealing with large numbers of urines daily, and it required only the simplest equipment.

A method involving hatching and quantitative assessment of miracidia and dead eggs, which excluded microscopy, would be a great improvement on present crude techniques, in which many valuable technician-hours are spent on light-microscopy. Nephelometry, turbidimentry, electronic counting procedures, or labelling a specimen with a radio-active substance taken up by eggs, may offer a fruitful field for further work.

Comparison of recipient groups

The results of the comparable investigations performed on the three treatment groups A, B and C, shown in Tables 3, 4 and 5 demonstrate that the groups were similar in respect of all important variables likely to influence treatment.

The high incidence of multiple parasitic infections in these patients was noteworthy. In group A, 76.6% of 60 patients harboured multiple parasites other than *S. haematobium*, representing 70 other infections. One patient had pulmonary tuberculosis with cavitation and a positive sputum. In group B 84% of 50 patients harboured multiple parasites other than *S. haematobium* representing 59 other infections. One patient had secondary syphilis and 2 had chancroid. In group C, 84% of 50 patients harboured multiple parasites other than *S. haematobium* representing 71 other infections. One patient had a consolidated upper lobe and one patient had a paraphimosis on admission. The acute conditions were treated before commencing schistosomicidal therapy. The patient with pulmonary tuberculosis was given specific chemotherapy concurrently with a course of antimony.

TABLE 4

PARASITIC CONDITIONS OCCURRING IN PATIENTS IN THE THREE TREATMENT GROUPS

Parasite	Treatment group		
	A	B	C
Ova of hookworm	28	16	20
Ova of *Schistosoma mansoni*	5	4	3 [a]
Ova of *Taenia* spp.	5	1	3
Ova of *Ascaris lumbricoides*	4	3	2
Ova of *Trichuris trichiura*	2	2	3
Larvae of *Strongyloides stercoralis*	5	5	5
Cysts of *Entamoeba histolytica*	4	6	7
Trophozoites of *Plasmodium falciparum*	5	6	11
Trophozoites and schizonts of *P. malariae*	–	1	2
Microfilariae of *Wuchereria bancrofti*	12	13	10
Microfilariae of *Acanthocheilonema perstans*	–	2	5

[a] Two patients had *S. mansoni* in urine.

The type of parasitic infections encountered, in addition to *S. haematobium*, is shown in Table 4. A broad spectrum of infecting parasites was seen with a high incidence of hookworm infection, malaria and bancroftian microfilaraemia.

Pretreatment egg counts

The relative frequency distribution of the pre-treatment egg counts in each group is shown in Table 5. This demonstrated the typical positive skew pattern of counts commonly seen in surveys of large numbers of patients with urinary schistosomiasis.

TABLE 5
RELATIVE FREQUENCIES OF EGG NUMBERS COUNTED IN RANDOM SAMPLES OF URINE FROM ADULT MALES WITH URINARY SCHISTOSOMIASIS [a]

Eggs per 10 ml of urine	Group A (60 patients)						Group B (50 patients)						Group C (50 patients)					
	Casual specimens			2 timed specimens			Casual specimens			2 timed specimens			Casual specimens			2 timed specimens		
	No.	%	Σ% [b]	No.	%	Σ% [b]	No.	%	Σ% [b]	No.	%	Σ% [b]	No.	%	Σ% [b]	No.	%	Σ% [b]
1-99	22	37	37	61	51	51	26	52	52	46	46	46	24	48	48	53	53	53
100-199	5	8	45	17	14	65	8	16	68	20	20	66	10	20	68	13	13	66
200-299	6	10	55	6	5	70	1	2	70	12	12	78	4	8	76	9	9	75
300-399	6	10	65	4	3	73	1	2	72	5	5	83	3	6	82	5	5	80
400-499	2	3	68	2	2	75	5	10	82	6	6	89	2	4	86	3	3	83
500-599	3	5	73	6	5	80	2	4	86	3	3	92	3	6	92	2	2	85
600-699	2	3	76	4	3	83	1	2	88	—	0	92	—	0	92	2	2	87
700-799	2	3	79	5	4	87	—	0	88	3	3	95	—	0	92	2	2	89
800-899	1	2	81	1	1	88	1	2	90	1	1	96	—	0	92	3	3	92
900-999	2	3	84	3	3	91	—	0	90	—	0	96	1	2	94	2	2	94
1 000-1 099	1	2	86	4	3	94	—	0	90	—	0	96	—	0	94	3	3	97
1 100-1 199	3	5	91	—	0	94	1	2	92	1	1	97	—	0	94	1	1	98
1 200-1 299	1	2	93	1	1	95	—	0	92	1	1	98	—	0	94	—	0	98
1 300-1 399	—	0	93	3	3	98	1	2	94	1	1	99	—	0	94	—	0	98
1 400-1 499	1	2	95	—	0	98	—	0	94	—	0	99	—	0	94	—	0	98
1 500-1 599	—	0	95	1	1	99	1	2	96	—	0	99	—	0	94	—	0	98
1 600-1 699	—	0	95	—	0	99	1	2	98	—	0	99	2	4	98	—	0	98
1 700-1 799	—	0	95	1	1	100	—	0	98	—	0	99	—	0	98	—	0	98
1 800-1 899	1	2	97	1	1	101	—	0	98	—	0	99	—	0	98	—	0	98
1 900-1 999	—	0	97	—	0	101	—	0	98	—	0	99	1	2	100	—	0	98
2 000-2 099	1	2	99	—	0	101	—	0	98	—	0	99	—	0	100	—	0	98
2 100-2 199	—	0	99	—	0	101	—	0	98	1	1	100	—	0	100	—	0	98
2 200-2 299	—	0	99	—	0	101	—	0	98	—	0	100	—	0	100	—	0	98
2 300-2 399	—	0	99	—	0	101	—	0	98	—	0	100	—	0	100	—	0	98
2 400-2 499	—	0	99	—	0	101	—	0	98	—	0	100	—	0	100	—	0	98
>2 500 [c]	1	2	101	—	0	101	1	2	100	—	0	100	—	0	100	2	2	100
Total	60		101	120		101	50		100	100		100	50		100	100		100

[a] Counts from a random 10-ml sample from one casual specimen taken near noon and from 2 random samples from the same patients drawn from hospital collections made between 10 a.m. and 2 p.m.
[b] Cumulative percentage. [c] Counts of 2550, 2633, 2734 and 6195 eggs per 10 ml of urine.

Excretion urography

Patients were submitted to intravenous pyelography when time was available in a busy hospital radiological department, and no case-selection was adopted. Table 6 shows the high incidence of pyelographic abnormality, 21% of the total number of patients and 34% of the sample, and the similarity of proportions of abnormalities in each treatment group.

The type of pyelographic abnormality is shown in Table 7. The major damage was ureteric and ranged from "beading" to gross hydroureter.

TABLE 6

INCIDENCE OF PYELOGRAPHIC ABNORMALITY IN SAMPLES OF ADULTS WITH *S. HAEMATOBIUM* INFECTION

Treatment group	Total number of patients in group	Number (and percentage) of intravenous pyelograms performed	Number (and percentage) abnormal	Number (and percentage) normal	Number (and percentage) not examined by pyelography
A	60	33 (55)	13 (22)	20 (33)	27 (45)
B	50	33 (66)	12 (24)	21 (42)	17 (34)
C	50	34 (68)	9 (18)	25 (50)	16 (32)
Total	160	100 (63)	34 (21)	66 (41)	60 (38)

$\chi^2 = 3.3476$ with 4 degrees of freedom; $0.7 > P > 0.5$; not significant

TABLE 7

TYPES OF ABNORMALITY FOUND IN 100 INTRAVENOUS PYELOGRAMS IN ADULT MALES WITH URINARY SCHISTOSOMIASIS

Abnormality			No. of times found in Group A[a]	No. of times found in Group B[b]	No. of times found in Group C[c]
Bladder calcification			7	3	5
Ureteric calcification			—	1	—
Irregular dilatation and narrowing ("beading") of lower ureter		Unilateral	4	8	4
		Bilateral	3	3	1
Hydroureter	Slight	Unilateral	3	3	1
		Bilateral	—	1	—
	Gross	Unilateral	2	—	2
		Bilateral	—	1	—
Hydronephrosis		Unilateral	1	—	1
		Bilateral	2	5	1
Non-excreting kidney			—	—	—
Stone			—	1	—
Filling defect in bladder			3	4	—
Other abnormalities			Duplex kidney		Bladder diverticulum. Horseshoe kidney

[a] 33 pyelograms. [b] 33 pyelograms. [c] 34 pyelograms.

Bladder calcification was seen in 15% of pyelograms and hydronephrosis in 10%. The sample was drawn from an adult male population some 30%–40% of whom had a history of infection of over 5 years' duration. In no patient was an abnormal blood urea level found.

The association between the occurrence of radiological abnormality and the incidence of high urinary egg-counts was investigated. Counts of under 200 ova per 10 ml of urine accounted for 305 of a total 480 pretreatment counts, 63% (Table 5), and this level was a convenient dividing point. The egg-ouput of radiological positive cases was compared with that of radiological negative cases and the results (Table 8) demonstrated that there

TABLE 8

COMPARISON OF PROPORTIONS OF EGG COUNTS IN RADIOLOGICAL POSITIVE AND NEGATIVE CASES

Radiological result	Counts over 200 ova per random 10 ml of urine	Counts under 200 ova per random 10 ml of urine	
Positive	58	44	102
Negative	60	138	198
Totals	118	182	300

$x^2 = 18.8$ with 1 degree of freedom; $P < 0.001$

was a highly significant association between the proportions of radiological positive cases and the occurence of high pretreatment egg-counts, which carries urgent therapeutic implications.

Controls

Although non-treated or dummy controls would have been ideal, under the circumstances it was decided to use the patient, when possible, as his own control.

The criterion of cure was to be the absence of eggs from the urines on follow-up over a period of 3 months. No alternative proved methods of evaluating cure exist (WHO Scientific Group on Chemotherapy of Bilharziasis, 1966). Previous longitudinal studies of egg-excretion in *S. haematobium* disease had indicated that egg output was fairly constant over some months (Barlow & Meleney, 1949; Stimmel & Scott, 1956; Bradley, 1962; Standen, 1962). There was strong indirect evidence that continuous absence of eggs from the urine of a treated patient is overwhelmingly more likely to be due to treatment than to the spontaneous cessation of egg-passage. To confirm this, a patient's egg-count on his casual urine screening specimen was compared with his 2 timed egg-counts on admission to hospital. Comparison on a group basis was valid because the same method of urine examination was applied to the same random volume of casual and hospital specimens collected at the time of maximum egg output, and in no instance was a schistosomicide given between screening and admission to hospital.

In each of the three treatment groups, those patients who were treated 4–7 weeks after initial screening were termed " 1-month controls ". Those patients treated between 8 and 11 weeks after primary screening were accepted as " 2-month controls " and patients treated at 12 or more weeks after screening were designated " 3-month controls ". During the first 2 or 3 days in hospital, no attempts were made to restrict activity and patients were fully ambulant. Comparison of the egg-output was made by a non-parametric, two-way analysis of variance by ranks (Friedman, 1937, 1940). Samples were related in being from the same patient and the test was thought to have a high power-efficiency. The null hypothesis was that the egg-counts at different times in the same individuals were drawn from the same population.

The statistic computed in this test is χr^2 which has a sampling distribution approximated by the χ^2 distribution with $k-1$ degrees of freedom, where k is the number of columns, i.e., k conditions. Table 9 shows the value of χr^2 and the probability under the null hypothesis computed separately for each group of controls at each month. When the number of rows was small, exact probability tables were used.

In 7 cells the null hypothesis that the egg counts came from the same population was retained, i.e., any difference existing could be attributed to random sampling variation. However, in 2 cells, those of group A at 1 and 2 months, there was a significant difference in the populations of egg-counts at the different periods of time. These counts were reflected in the over-all analysis made of the pooled controls, as shown in Table 10.

The probable true state of affairs was reflected in the counts at 3 months. The individual probabilities in the cells of group A, at 1 and 2 months, had contributed to the over-all significant difference in egg-counts at these times in the analysis of the

TABLE 9

PROBABILITY THAT URINE SAMPLES, TAKEN UNDER SIMILAR CONDITIONS FROM THE SAME PATIENTS WITH URINARY SCHISTOSOMIASIS, AT 1-3 MONTH INTERVALS, CONTAIN SIMILAR NUMBERS OF EGGS [a]

Group	1-month controls			2-months controls			3-months controls		
	xr^2	N [b]	Probability [c]	xr^2	N [b]	Probability [c]	xr^2	N [b]	Probability [c]
A	16.1	21	$P < 0.001$ [d]	8.4	5	Ex. P [e] $= 0.0085$ [d]	0.0	4	Ex. P [e] $= 1$ (NS) [f]
B	1.385	13	$0.7 > P > 0.5$ (NS) [f]	5.43	7	Ex. P [e] $= 0.085$ (NS) [f]	0.808	13	$0.7 > P > 0.5$ (NS) [f]
C	0.875	12	$0.7 > P > 0.5$ (NS) [f]	0.875	4	Ex. P [e] $= 0.931 > P > 0.653$ (NS) [f]	0.4	15	$0.9 > P > 0.8$ (NS) [f]

[a] Calculated from xr^2 on a null hypothesis that egg counts on the same individuals at different periods in the absence of treatment arose from the same population. Each category has 2 degrees of freedom.

[b] Number of patients accepted as controls (and hence the number of series of egg-counts).

[c] Under the null hypothesis. [d] Highly significant with rejection of the null hypothesis.

[e] Exact probability when N = 10 (Table N, Siegel, 1956). [f] Not significant.

TABLE 10

PROBABILITY THAT URINARY EGG COUNTS FROM THE SAME PATIENTS AT DIFFERENT TIME INTERVALS IN THE ABSENCE OF TREATMENT CAME FROM THE SAME POPULATION [a]

Control	Total No.	xr^2	Degrees of freedom	Probability
1-month	46	10.4	2	$0.01 > P > 0.001$ [b]
2-month	16	8.0	2	$0.02 > P > 0.01$ [c]
3-month	32	0.422	2	$0.9 > P > 0.8$ [d]

[a] Analysis of pooled controls from Table 9.

[b] Highly significant.

[c] Significant

[d] Non-significant.

pooled controls. The rationale on which this test is based is one on which the egg-counts are ranked horizontally, i.e., the samples are ranked under different conditions, which in this case is the time of measurement. If the samples came from the same population, then the distribution of ranks in each column would be a matter of chance, and the sums of the ranks in each column would be roughly equal.

Investigation of the discrepancy in the 2 cells of group A controls in Table 9 revealed a human error in that a technician in training was producing low egg-counts initially. This was rectified and later assessment showed no significant inter-technician difference. This finding emphasized the constant presence of observer error which can be minimized only by familiarity with the chosen technique.

The most significant finding in the control studies was that in 94 patients subjected to repeated egg-counts at intervals of 1, 2 or 3 months, eggs never disappeared from the 10-ml randomly sampled specimens of urine. Seven cells in Table 9 showed that no significant difference existed between the populations of egg counts from the same patients at intervals of from 1 to 3 months in the absence of treatment. These studies demonstrated that, if a therapeutic assessment was made 2 or 3 months after treatment, disappearance of eggs could be fairly attributed to therapy, and not to spontaneous cessation of egg-passage.

The three treatment groups, composed of randomly allocated patients found during screening, were comparable in respect of pretreatment egg-output, body-weight and clinical findings. Secular fluctuations in egg-excretion would not falsify therapeutic assessment. Hence observations on drug toxicity and therapeutic effect would be valid.

TREATMENT

Current opinion on antimonial drugs may be summarized as follows:

(1) Potassium or sodium antimony tartrate is the most effective preparation in the treatment of schistosomiasis and the most toxic.

(2) Trivalent sodium antimonylgluconate is less effective and less toxic.

(3) Antimony dimercaptosuccinate, while highly effective, is the subject of conflicting reports with regard to dosage and toxicity.

(4) Other intramuscular antimonials are less effective but less toxic than the antimonials mentioned.

These generalizations are based upon the results of drug trials in which, all too commonly, only one preparation has been used. Comparisons between antimonials used in different trials become invalid because the dosage schemes have been so variable and the amount of metallic antimony required for cure has been obscured. Standen (1963) remarked " After 40 years extensive use of trivalent antimony no apparent rationale in treatment has developed ". Indeed, much antimonial chemotherapy is almost mediaeval in concept, and cure rates tend to vary with individual or group tolerance to the amount of injected metal, rather than with other more clearly defined criteria. This is due to a basic lack of knowledge of antimonial action and metabolism, and until this is corrected, recommendations for optimal antimonial use will continue to be on a trial-and-error basis. In the present trials it was hoped to acquire basic information about tolerance, toxicity and cure rates in a young adult working population by selecting a dose of metallic antimony thought to give satisfactory cure rates, and comparing it with two other preparations of antimony. To eliminate other variables the recommended dosage schemes of the different schistosomicides were adjusted so that equivalent amounts of antimony were given over the same period.

Three antimonial preparations, sodium antimonylgluconate (TSAG), sodium antimony (III) meso-2, 3-dimercaptosuccinate (TWSb) and antimony sodium tartrate (AST), were selected for comparison.

Sodium antimonylgluconate (Triostam) (TSAG)

This drug contains 36% of trivalent antimony metal. The contents of one ampoule (190 mg) were dissolved in 3.5 ml of ice-cold, sterile, distilled water giving a solution of strength of approximately 54.3 mg TSAG per ml. Solutions were freshly prepared each day immediately before use and any unused solution was discarded. The course was given intravenously daily as shown in Table 11. Thus, the total dose for adult working males was 1.466 g of sodium antimonylgluconate, approxi-

TABLE 11

DOSAGE SCHEME USED FOR SODIUM ANTIMONYLGLUCONATE TREATMENT OF ADULT MALES WITH URINARY SCHISTOSOMIASIS

Day	TSAG (ml)	TSAG (mg)	Cumulative TSAG (ml)	Cumulative TSAG (mg)	Antimony content of dose (mg)	Cumulative amount of antimony (mg)
1	0.5	27.15	0.5	27.15	9.75	9.75
2	1.0	54.3	1.5	81.45	19.5	29.25
3	1.5	81.45	3.0	162.9	29.25	58.5
4	2.0	108.6	5.0	271.5	39.0	97.5
5	2.0	108.6	7.0	380.1	39.0	136.5
6	2.0	108.6	9.0	488.7	39.0	175.5
7	2.0	108.6	11.0	597.3	39.0	214.5
8	2.0	108.6	13.0	705.9	39.0	253.5
9	2.0	108.6	15.0	814.5	39.0	292.5
10	2.0	108.6	17.0	923.1	39.0	331.5
11	2.0	108.6	19.0	1 031.7	39.0	370.5
12	2.0	108.6	21.0	1 140.3	39.0	409.5
13	2.0	108.6	23.0	1 248.9	39.0	448.5
14	2.0	108.6	25.0	1 357.5	39.0	487.5
15	2.0	108.6	27.0	1 466.1	39.0	526.5

mately equivalent to 527 mg of trivalent antimony, given intravenously in 15 days.

Sodium antimony dimercaptosuccinate (Astiban) (TWSb)

This drug contains 25%–26% of trivalent antimony metal. The contents of one 2.0-g vial were dissolved in 20 ml of sterile distilled water giving a 10% solution of strength 100 mg TWSb per ml, approximately equivalent to 25 mg of antimony per ml. The course was given intramuscularly daily as shown in Table 12. The total dose for adult working males was 2.12 g of antimony dimercaptosuccinate, approximately equivalent to 530 mg of trivalent antimony, given intramuscularly in 15 days.

Antimony sodium tartrate (AST)

This drug contains 39.3% of trivalent antimony metal. A 6% solution (w/v) was used containing 60 mg of AST per ml and approximately 23.6 mg of antimony per ml. Solutions were prepared fresh each day, immediately before use, with double glass-distilled water and any unused solution was discarded. The course was given intravenously daily as shown in Table 13. The total dose for adult working males was 1.347 g of 6% AST solution, approximately equivalent to 529.5 mg of trivalent antimony, given intravenously over a period of 15 days.

Comments on the use of sodium antimonylgluconate

The manufacturer's recommendations on dosage range from 12 mg to 17 mg per kg of body-weight, given intravenously on 6 consecutive days. The majority of our patients weighed about 60 kg, and hence could have received 720 mg of TSAG (approximately 259 mg of antimony) at the lower level, or 1020 mg of TSAG (approximately 367 mg of antimony) at the upper level.

A second method of calculation of dosage is given as 0.5 ml of a standard solution (containing 190 mg of TSAG in 3.5 ml of water) for every 10 kg of body-weight. Thus a 60-kg man could receive 3 ml per day for 6 days, equivalent to 977 mg of TSAG or 351 mg of antimony.

The manufacturer also states that in heavy infections it may be considered preferable to extend the course to 12 days, using the same daily dosage. In a heavy infection (the criteria for the diagnosis of which are not given), a 60-kg man could receive 1954 mg of TSAG, equivalent to 702 mg of antimony in 12 days.

There is thus a wide range of recommended dosages which may account partly for variation in

TABLE 12
DOSAGE SCHEME USED FOR ANTIMONY DIMERCAPTOSUCCINATE TREATMENT OF ADULT MALES WITH URINARY SCHISTOSOMIASIS

Day	TWSb (ml)	TWSb (mg)	Cumulative TWSb (ml)	Cumulative TWSb (mg)	Antimony content of dose (mg)	Cumulative amount of antimony (mg)
1	0.4	40	0.4	40	10	10
2	0.8	80	1.2	120	20	30
3	1.2	120	2.4	240	30	60
4	1.6	160	4.0	400	40	100
5	1.6	160	5.6	560	40	140
6	1.6	160	7.2	720	40	180
7	1.6	160	8.8	880	40	220
8	1.6	160	10.4	1 040	40	260
9	1.6	160	12.0	1 200	40	300
10	1.6	160	13.6	1 360	40	340
11	1.6	160	15.2	1 520	40	380
12	1.6	160	16.8	1 680	40	420
13	1.6	160	18.4	1 840	40	460
14	1.6	160	20.0	2 000	40	500
15	1.2	120	21.2	2 120	30	530

TABLE 13
DOSAGE SCHEME USED FOR ANTIMONY SODIUM TARTRATE TREATMENT OF ADULT MALES WITH URINARY SCHISTOSOMIASIS

Day	AST (ml)	AST (mg)	Cumulative AST (ml)	Cumulative AST (mg)	Antimony content of dose (mg)	Cumulative amount of antimony (mg)
1	0.4	24	0.4	24	9.4	9.4
2	0.85	51	1.25	75	20.0	29.4
3	1.25	75	2.5	150	29.5	58.9
4	1.7	102	4.2	252	40.1	99.0
5	1.7	102	5.9	354	40.1	139.1
6	1.7	102	7.6	456	40.1	179.2
7	1.7	102	9.3	558	40.1	219.3
8	1.7	102	11.0	660	40.1	259.4
9	1.7	102	12.7	762	40.1	299.5
10	1.7	102	14.4	864	40.1	339.6
11	1.7	102	16.1	966	40.1	379.7
12	1.7	102	17.8	1 068	40,1	419.8
13	1.7	102	19.5	1 170	40.1	459.9
14	1.7	102	21.2	1 272	40.1	500.0
15	1.25	75	22.45	1 347	29.5	529.5

reported therapeutic results. The TSAG course of 1.466 g in 15 days (equivalent to 527 mg of antimony) was a compromise, being an amount higher than the manufacturer's recommended course for 6 days at the upper level of the first dosage calculation, but lower than the recommended amount for a 12-day course under the second method of dosage calculation.

The course used in these trials was given over a longer period of time than is usually recommended, but the present work aimed not at reduction of the length of treatment, but at the comparison of the effects of equivalent amounts of antimony in humans, using different preparations given over the same period.

Comments on the use of sodium antimony dimercaptosuccinate

The course employed was a standard total dosage. Early work on this drug confirmed the superiority of the intramuscular to the intravenous route. Times of administration have varied from 1 to 14 days. Opinion in the last three years has favoured the intramuscular administration of the drug only two or three times weekly; the present work confirmed this opinion. On a mg/kg/day basis, for a 60-kg man, the total dose was approximately 35 mg per kg.

Comments on the use of antimony sodium tartrate

The course used was a standard one (e.g., Christopherson & Newlove, 1919; El Halawani, 1962; British Pharmaceutical Codex, 1963). The antimony content was higher than that used by some authorities (e.g., El Halawani, 1962), although lower than that given in some European centres (e.g. Khalil, Rifaat & Woodruff, 1962). It was given over a shorter period than is usual in the classical course because there is evidence that better results are obtained than when it is given on alternate days (Fairley, 1951).

Humidity affects antimony sodium tartrate; all AST used at this centre was dried at 105°C for 12 hours and kept over silica gel in an air-conditioned room to maintain the specifications shown in the dosage schedule. Although variation in antimony content of courses may be a minor contributory factor in explaining slight variations in clinical results in different geographical areas, differing immune status of recipients, egg-output and nutritional state are all likely to play a greater part.

It was thought that the total amount of metallic antimony given to comparable recipient groups by means of the three different courses was within the limits of clinical and experimental error and could

reasonably be considered identical. All drug preparation and administration was done personally.

Clinical data

A total of 39% of all patients failed to complete the prescribed treatment; the amounts of metallic antimony they received is as follows: In group A treated with intravenous TSAG, 58 out of 60 patients completed the course. The 2 patients who failed to complete the course received less than 400 mg of antimony. In group B, 50 patients were treated with intramuscular TWSb, 24 completed the course, 26 failed to do so and of these 12 received more than 400 mg of antimony and 14 received less than 400 mg. Of the 50 patients in group C treated with intravenous AST only 15 completed the course, 35 failed to complete the course and 14 received more than 400 mg of antimony while 21 received less than 400 mg. The details of the incomplete courses are indicated in Table 14 which shows that patients receiving AST, in general, tolerated smaller amounts of metallic antimony than did patients receiving the other two preparations.

Of the three drugs, TSAG was most acceptable to the patients. Treatment was terminated by the physician for obvious reasons such as hepatitis, repeated severe vomiting, skin rashes or pneumonia, while patient refusal was commonly due to excessive vomiting.

Tolerance

There was a marked difference in tolerance to the three antimonial preparations as is shown in Table 15. Failure to complete the course owing to severity of

TABLE 14

DETAILS OF INCOMPLETE COURSES OF ANTIMONY TREATMENT OF THE THREE GROUPS OF PATIENTS

Number of patients	Amount of drug (mg)	Period of treatment (days)	Approximate equivalent in metallic antimony (mg)
	Group A (TSAG)		
1	488.7	6	175
1	923.1	10	331
	Group B (TWSb)		
3	2 000	15	500
2	1 840	15	460
7	1 680	12	420
7	1 520	11/12	380
6	1 360	12	340
1	880	7	220
	Group C (AST)		
6	1 272	15/18	500
5	1 170	15/18	460
3	1 068	15/16	420
7	966	15/16	380
4	864	10/11	340
2	762	9/10	300
3	660	8/9	260
3	558	7/8	220
1	456	6	180
1	252	6	99

TABLE 15

PATIENT TOLERANCE OF THREE ANTIMONIAL PREPARATIONS GIVEN IN COURSES CONTAINING EQUAL AMOUNTS OF METALLIC ANTIMONY

Group, treatment and number of patients	Completed course in:			Total and percentage	Incomplete course		Total and percentage
	15 days	16 days	17 days		Terminated by physician	Refused by patient	
Group A; TSAG; 60	57	1	—	58 (97)	2	—	2 (3)
Group B; TWSb; 50	21	1	2	24 (48)	10	16	26 (52)
Group C; AST; 50	5	5	5	15 (30)	26	9	35 (70)
Total	83	7	7	97	38	25	63

side-effects was as follows: TSAG, 2 patients out of 60 (3.3%); TWSb, 26 out of 50 (52%); AST, 35 out of 50 (70%).

Incidence of side-effects

The incidence of side-effects in the three groups is shown in Table 16. Only 22% of all patients were free from side-effects during treatment. Those tabulated were complaints which were persistent and vociferous, or when objective confirmation was available. Complaints were frequently multiple. Table 17 shows the types of side-effects encountered with the three preparations.

The mere cataloguing of the incidence and types of side-effects gives little indication of the qualitative aspects of the toxicity of antimonial drugs. Gastrointestinal symptoms predominated, and were marked in those patients receiving intramuscular TWSb or intravenous AST. Vomiting was most severe and exhausting in those receiving the intramuscular preparation, and anorexia was profound, being associated with constant nausea aggravated by the sight or smell of food. Arthralgia was common to all

TABLE 16

INCIDENCE OF SIDE-EFFECTS DURING TREATMENT OF URINARY SCHISTOSOMIASIS WITH THREE DIFFERENT ANTIMONIAL PREPARATIONS AT EQUIMETALLIC DOSAGE

Group, treatment and number of patients	Number (and percentage) with no side-effects		Number (and percentage) with side-effects	
Group A; TSAG; 60	30	(50)	30	(50)
Group B; TWSb; 50	3	(6)	47	(94)
Group C; AST; 50	2	(4)	48	(96)
Total	35	(22)	125	(78)

TABLE 17

SIDE-EFFECTS ENCOUNTERED DURING TREATMENT OF URINARY SCHISTOSOMIASIS WITH THREE DIFFERENT ANTIMONIAL PREPARATIONS

Side-effect	Group A; TSAG (60 patients)	Group B; TWSb (50 patients)	Group C; AST (50 patients)
Anorexia	—	42	26
Nausea	—	34	23
Vomiting	1	32	32
Abdominal pain	7	6	10
Metallic taste	—	5	—
Loose stools or diarrhoea	—	3	9
Tender hepatomegaly	1	1	—
Urobilinuria	1	1	1
Haematemesis	—	—	1
Substernal pain	4	11	12
Acute vascular collapse	—	—	3
Cough during injection	1	—	3
Pneumonia during treatment	—	—	2
Generalized muscle pain	3	14	4
Arthralgia	15	17	17
Frozen shoulder	—	—	4
Skin rash	2 (1 post-antimonial zoster)	—	5
Epididymitis/funiculitis/orchitis	3	1	—
Pyrexial reaction	3 (2 associated with IVP)	2	5
Depression	—	5	4
Prostration	—	5	8
Thrombophlebitis of arm	—	—	1
Local pain at injection site	—	9	—
Multiple boils	1	—	—

groups although patients in groups B (TWSb) and C (AST) tended to complain to a greater extent, presumably due to their already depressed state from gastrointestinal toxicity.

The frozen shoulder syndrome occurring in group C (AST) was of interest. Complete immobility of a shoulder joint occurred with extreme pain on movement. It occurred equally in the arm used for injection or in the other arm and was not associated with thrombophlebitis, which was seen only once. When AST was withheld movement was regained in 3–5 days, but if antimony treatment was restarted, the syndrome reappeared.

Cardiovascular complications of antimonial treatment have received much attention in the past. Two recognizable syndromes are generally held to occur. One pattern involves the heart primarily with the development of a cardiac arrhythmia, often terminated by death. In the other a shock-like pattern ensues having many of the characteristics of anaphylaxis (WHO Scientific Group on Research in Bilharziasis (Chemotherapy), 1966). In this series, substernal pain was fairly common and 3 cases of acute vascular collapse occurred (1.9%), all in patients receiving intravenous antimony sodium tartrate. The weights of the patients were 45 kg, 55.5 kg and 68 kg, and there were no contraindications to antimony treatment. All the attacks were characterized by the sudden onset of central chest pain with vertigo, hypotension, a thready peripheral pulse, and autonomic accompaniments of nausea, apprehension and sweating. One attack occurred immediately after the first intravenous injection and the others on the eighth and twelfth day, after 219 mg and 351 mg of metallic antimony had been given. In no case was the injection given rapidly and all recovered after simple rest in bed. This clinical state resembled an anaphylactic response.

The well-known complication of cough during the injection was not a problem but consolidation of a lung segment occurred twice. In each case the left lower lobe was involved with pyrexia, purulent sputum and typical physical and radiological signs of a lobar consolidation. In neither patient was there an increase in eosinophils suggesting death of adult worms from a lung shift and both responded to antibiotics. They were regarded as intercurrent bacterial pneumonias.

A skin rash during treatment occurred in 6 of 160 patients (3.8%) The rash commenced with a fine papular or mammilarial eruption of the face and the backs of the hands, spreading later to the trunk. Two patients had branny desquamation of the skin, and 2 patients showed urticarial weals. Because of the risk of exfoliative dermatitis, any rash was considered a contra-indication to further treatment and regression of symptoms was rapid. One patient developed typical herpes zoster 4 days after treatment.

Four patients (2.5%) developed acute funiculitis, epididymitis or orchitis with pyrexia and pain. The condition was seen between 9 and 14 days after the start of antimony treatment of patients with heavy infections of *Wuchereria bancrofti* in their pretreatment night blood films. Death of adult nematodes following trivalent antimony may have been the explanation, but equally so may death of adult trematodes. The condition was not seen in the other patients in the series with pretreatment microfilaraemia.

The incidence of major gastrointestinal toxicity in groups B (TWSb) and C (AST), with the secondary phenomena of profound depression and weakness caused by severe anorexia, nausea and vomiting was responsible for the high refusal rate of patients to complete these courses of treatment.

Antimony is potentially hepatotoxic and caution is necessary in its use in populations subject to malnutrition with possible latent hepatic dysfunction. In these trials, three examples of acute biochemical disturbance occurred (1.9%).

Electrocardiography during treatment

Daily electrocardiograms were recorded on as many patients as possible, using a direct-writing machine, employing the standard leads I, II, III, the augmented unipolar limb leads aVR, aVL, aVF (Goldberger, 1953), and chest leads V_1–V_6 (American Heart Association, 1938). The features analysed and the criteria of normality adopted have been described previously (Davis, 1961).

The major changes in the electrocardiogram induced by antimony are alteration in the shape of the ST segment, flattening or inversion of the T wave, lengthening of the QT_c, and, rarely, the appearance of a ventricular dysrthythmia. The degree and extent of change varies and depends on a number of factors, the most important of which are racial origin and the type of antimonial preparation. Reports on the electrocardiographic abnormalities caused by the three drugs used in the present trial have been presented recently by Rowland (1956), Honey (1960) and Davis (1961).

Grading of electrocardiographic changes for analysis poses many difficulties (for discussion see

Honey, 1960). In this series only a complete flattening or frank inversion of a T wave was accepted as abnormal.

Table 18 shows that, at equivalent antimonial dosage in comparable groups of patients, there was no significant difference in the incidence of T-wave

TABLE 18

T-WAVE CHANGES IN PATIENTS WITH URINARY SCHISTOSOMIASIS GIVEN A COMPLETE COURSE OF ONE OF THREE ANTIMONIAL PREPARATIONS AT EQUIVALENT METALLIC DOSAGE

Antimonial preparation	No. (and percentage) showing change in T wave in ECG	No. (and percentage) showing no change in T wave in ECG	Total
TSAG	21 (68)	10 (32)	31
TWSb	10 (42)	14 (58)	24
AST	10 (67)	5 (33)	15
Total	41 (59)	29 (41)	70

$\chi^2 = 4.396$ with 2 degrees of freedom; $0.2 > P > 0.1$ (not significant)

changes. Neither the incidence nor the extent of T-wave change was related to the amount of antimony given. The greatest number of changes occurred after AST and the least number after TWSb. No dysrhythmias were detected and the electrocardiographic signs were not associated with any symptoms other than substernal pain. There was no lower limit of dosage at which these signs appeared. In individual patients there tended to be a steady progression of T-wave change with continued treatment.

The mechanism of the changes is conjectural but there is experimental and clinical evidence (Franz, 1937; Bradley & Fredrick, 1941) that they are due to cumulation of antimony in the myocardium producing altered function, and possibly even superficial myocardial damage. The higher incidence of T-wave changes associated with the intravenous antimonials suggested that the myocardium may be affected more by the high, although transient blood levels occurring during the administration of these drugs. It was concluded that individual susceptibility may play an important role in the production of cardiographic change. A shock-like syndrome having the characteristics of an anaphylactic response occurs during antimonial treatment, and such responses are well recognized with many other therapeutic compounds.

POST-TREATMENT OBSERVATIONS

Follow-up procedure

Follow-up urine examinations were performed, by the method described, for 5 successive days until it became clear that examination for 3 successive days was adequate. As already described, each patient was allocated his own glassware to eliminate the possibility of contamination. Follow-up results were expressed as:

C = cure; no miracidia or ova in any specimen.

PC = possible cure; no miracidia but dead ova seen. Dead ova may be of recent origin as previously defined, or may be black, shrivelled or distorted.

F = failure; the presence of hatched miracidia.

Results were expressed on an all-or-none basis. To qualify as a cure, a patient had a completely negative urine each day of follow-up. The presence of only 1 dead egg in any one specimen relegated the patient to the category of possible cure. The presence of 1 hatched miracidium in a urine placed the case in the failed category and examinations were continued to check the finding.

Therapeutic results

The results of treatment in 97 patients who received a complete course of an antimonial drug (530 mg of metallic antimony) are shown in Table 19. The results of patients who received incomplete courses are shown in Tables 20 and 21. Table 20 gives details of 26 patients who received a total dose of more than 400 mg of metallic antimony and Table 21 the details of 37 patients whose total dosage was less than 400 mg of antimony.

Table 22 shows the month when miracidia were first detected in the urine during the 1 year after treatment. The numbers of failures in Tables 19, 20 and 21 do not necessarily correspond with those in Table 22 because occasional patients, known to be passing miracidia at 1 month, were missed at a subsequent examination.

Comment

All patients passing eggs which hatched at any time after treatment were regarded as failures. In those given a complete course of an antimonial, 1 patient in each treatment group missed all post-treatment examinations. In those given a total dose of over 400 mg of antimony 1 patient from the TWSb and AST groups missed all follow-ups.

TABLE 19

THERAPEUTIC RESULTS IN 97 PATIENTS GIVEN A COMPLETE COURSE [a] OF AN ANTIMONIAL DRUG

Months after treatment	Group A; TSAG [b] (58 patients)				Group B; TWSb [c] (24 patients)				Group C; AST [b] (15 patients)				Total				Total
	C [d]	PC [e]	F [f]	Lost	C [d]	PC [e]	F [f]	Lost	C [d]	PC [e]	F [f]	Lost	C [d]	PC [e]	F [f]	Lost	
1	24	27	5	2	3	19	0	2	11	2	0	2	38	48	5	6	97
2	18	29	9	2	9	13	1	1	9	5	0	1	36	47	10	4	97
3	13	28	12	5	13	10	0	1	9	4	0	2	35	42	12	8	97
6	14	20	14	10	9	8	2	5	7	1	0	7	30	29	16	22	97
9	17	10	15	16	9	2	3	10	3	0	0	12	29	12	18	38	97
12	19	9	14	16	6	2	4	12	5	0	1	9	30	11	19	37	97

[a] 530 mg of metallic antimony. [b] Intravenous. [c] Intramuscular. [d] Cure; no miracidia or ova in any specimen of urine. [e] Possible cure; no miracidia, dead eggs only found. [f] Failure; miracidia isolated.

TABLE 20

THERAPEUTIC RESULTS IN 26 PATIENTS GIVEN AN INCOMPLETE COURSE [a] OF AN ANTIMONIAL DRUG

Months after treatment	Group A; TSAG [b]	Group B; TWSb [c] (12 patients)				Group C; AST [b] (14 patients)				Total				Total
		C [d]	PC [e]	F [f]	Lost	C [d]	PC [e]	F [f]	Lost	C [d]	PC [e]	F [f]	Lost	
1	No patients in this subgroup	5	5	0	2	5	8	0	1	10	13	0	3	26
2		5	3	2	2	8	5	0	1	13	8	2	3	26
3		7	1	2	2	5	7	1	1	12	8	3	3	26
6		7	1	2	2	10	2	1	1	17	3	3	3	26
9		5	3	2	2	3	2	0	9	8	5	2	11	26
12		7	0	1	4	6	2	0	6	13	2	1	10	26

[a] More than 400 mg of metallic antimony. [b] Intravenous. [c] Intramuscular. [d] Cure; no miracidia or ova in any specimen. [e] Possible cure; no miracidia, dead eggs only found. [f] Failure; miracidia isolated.

TABLE 21

THERAPEUTIC RESULTS IN 37 PATIENTS GIVEN AN INCOMPLETE COURSE [a] OF AN ANTIMONIAL DRUG

Months after treatment	Group A; TSAG [b] (2 patients)				Group B; TWSb [c] (14 patients)				Group C; AST [b] (21 patients)				Total				Total
	C [d]	PC [e]	F [f]	Lost	C [d]	PC [e]	F [f]	Lost	C [d]	PC [e]	F [f]	Lost	C [d]	PC [e]	F [f]	Lost	
1	0	1	1	0	3	7	0	4	6	11	2	2	9	19	3	6	37
2	0	0	2	0	6	4	1	3	4	12	3	2	10	16	6	5	37
3	0	1	1	0	6	1	3	4	8	6	5	2	14	8	9	6	37
6	0	0	2	0	1	5	3	5	5	5	6	5	6	10	11	10	37
9	0	0	2	0	3	1	3	7	1	2	0	18	4	3	5	25	37
12	0	0	2	0	2	3	2	7	4	3	2	12	6	6	6	19	37

[a] Less than 400 mg of metallic antimony. [b] Intravenous. [c] Intramuscular. [d] Cure; no miracidia or ova in any specimens of urine. [e] Possible cure; no miracidia, dead eggs only found. [f] Failure; miracidia isolated.

TABLE 22

TIME OF ONSET OF PASSAGE OF VIABLE OVA IN THE URINE OF PATIENTS AFTER ANTIMONIAL TREATMENT OF URINARY SCHISTOSOMIASIS

Months after treatment	Patients receiving complete course [a]			Patients receiving incomplete course [b]			Patients receiving incomplete course [c]		
	TSAG (58) [d]	TWSb (24) [d]	AST (15) [d]	TSAG (0) [d]	TWSb (12) [d]	AST (14) [d]	TSAG (2) [d]	TWSb (14) [d]	AST (21) [d]
1	5	0	0	0	0	0	1	0	2
2	5	1 [e], 1 [f]	0	0	2	0	1	1	1
3	4	0	0	0	0	1	0	2	2
6	4	2	0	0	0	0	0	0	2
9	2	0	0	0	0	0	0	0	0
12	0	1	1	0	0	0	0	0	0
Total number of failures seen during one year after treatment	20	4	1	0	2	1	2	3	7

[a] 530 mg of metallic antimony. [b] More than 400 mg of metallic antimony. [c] Less than 400 mg of metallic antimony.

[d] Total number of patients treated.

[e] This patient had 1 miracidium, on one occasion only, at 2 months. Miracidia appeared in greater numbers at 6 months. Examination at 3 months was negative.

[f] This patient had 1 miracidium, on one occasion, at 2 months. All other examinations over 1 year were negative (see text).

In patients given a total dose less than 400 mg of antimony, 3 of the TWSb group and 2 of the AST group missed all follow-ups. All other patients had satisfactory attendance rates after treatment although some missed a particular month owing to leave commitments. In calculating comparative cure rates for different subgroups those patients who missed all follow-up examinations were excluded.

Immediate therapeutic results following a complete course of an antimonial drug

For ease of analysis the results were considered as " immediate ", those of the first 3 months after treatment; and " late ", those at 6, 9 and 12 months. The immediate therapeutic results after a complete course confirmed the efficiency of all three preparations in treatment. The cumulative 3-month failure rate for sodium antimonylgluconate was 14 out of 57 (25%). Of these 14 failures, all of those examined at 6 months and 1 year remained positive and all except 1 showed over a 90% reduction of their pretreatment egg-load throughout the year. The pretreatment urinary egg-counts of these failures (14 patients, 42 counts, mean egg-output 657 ova per random 10 ml of a midday urine) were significantly higher (analysis of variance, $F = 42$ for 1 and 88 degrees of freedom; $P < 0.001$) than the pretreatment urinary egg-counts of the patients " cured ", i.e., consistently egg-free throughout early follow-up (16 patients, 48 counts, mean egg-output 80 ova per random 10 ml of midday urine).

Comparisons of lengths of histories and body-weights

These comparisons gave non-significant results. One patient given TWSb was found to have 1 miracidium in the urine on 1 occasion, at 2 months and was negative on 3 consecutive days at 3 months, but at 6 months miracidia appeared in greater numbers. In another patient in this subgroup, 1 miracidium was isolated once at the second month and is shown in Table 22. As this patient had 5 consecutive daily negative urines at 1 month, and was consistently negative from 3 months to 1 year, the validity of this isolated observation was doubted and it was ascribed to an error in the transcription of results. Thus the 3-month failure rate for antimony dimercaptosuccinate was 1 out of 23 (4%). There were no failures in the small number of patients who completed the course of antimony sodium tartrate.

The immediate therapeutic results of those given incomplete courses (Tables 20, 21) showed that failure rates increased as total dosage decreased. The cumulative proportion of failures at 3 months

in patients receiving a total dose of more than 400 mg of antimony was 3 out of 24 (12%) and in patients receiving a total dose less than 400 mg was 10 out of 32 (31%) (Table 22).

Late therapeutic results following a complete course of an antimonial drug

At 6 months the cumulative failure rate was 21 out of 94 (22%), the individual group failure rates being: TSAG, 18 out of 57 (32%); TWSb, 3 out of 23 (13%); and AST, 0 out of 14 (0%) (Table 22). At 1 year, failure rates increased over-all to 25 out of 94 (27%), individual cumulative failure rates being: TSAG, 20 out of 57 (35%); TWSb, 4 out of 23 (17%); and AST, 1 out of 14 (8%). These results do not represent absolute failure rates. The high loss and small size of sample introduced unknown variables and they should be regarded as guide-lines only.

Late therapeutic results following an incomplete course of an antimonial drug

These results are shown in Tables 20 and 21 and 23. In patients given a total dose of less than 400 mg of metallic antimony the over-all cumulative failure rate both at 6 months and 1 year was 12 out of 32 (38%). In patients receiving a total dose of more than 400 mg the anomalous failure rate of 3 out of 24 (12%) was attributed to the variation inherently present in small samples and the high loss rate. In these subgroups many of the known failures at 6 months were lost at 1 year and the true failure rate at this time is obviously higher.

Conditions in an endemic area

In an endemic area several points must be considered during follow-up.

(1) During the immediate (1–3 months) post-treatment period maturation of a preinfection may produce viable ova in the excreta. A preinfection is an infection acquired in the immediate weeks or days prior to the start of a course of treatment. The maturing forms of the parasite may be unaffected by treatment and egg-production may commence within 3 months. From the viewpoint of therapeutic results this would produce a false positive and bias against the drug.

(2) Maturation of a reinfection, i.e., an infection acquired immediately after treatment may, theoretically, result in egg-production in the immediate post-treatment period (1–3 months). The presence of ova in the excreta would be a false positive result and lead to bias against the drug.

(3) An inadequately treated infection, or an infection not cured by conventionally adequate treatment, will produce viable ova in the excreta indicating a true positive and denoting therapeutic failure.

(4) In theory any combination of (1), (2) and (3) may occur.

In addition, insensitive methods of excretal examination, or the incompetent use of a standard method, or examinations at infrequent intervals after treatment, may fail to detect viable ova.

Lack of knowledge of the natural history of the life of the egg in *S. haematobium* infections in humans precludes a rational approach to follow-up. Much of the biology of the egg in *S. mansoni* and *S. japonicum* infections has been studied in primates, but it would be unwise to extrapolate these observations to *S. haematobium* infections in man.

In an endemic area exposure may be constant, and the theoretical possibility of preinfection and reinfection makes it difficult to implicate any one cause when viable ova are detected within 6 months of treatment. The therapeutic results from the present trials throw some light on this confused state of affairs.

Optimum times of follow-up

It has always been difficult to decide when patients should be examined after treatment to enable a decision on the therapeutic efficiency of a drug to be made. The incubation period of *S. haematobium* disease in man, is unknown and estimates have ranged from 4 weeks to 2 years. It is unknown to what extent, if any, immune processes modify reinfections and their incubation periods. A clinical diagnosis of the early toxaemic stage of schistosomiasis (Katayama syndrome) is made extremely rarely in inhabitants of endemic areas and there are thus no clinical clues to the length of the incubation period. Because it is necessary to be clear about the meaning of " incubation period ", perhaps the term should refer to the time elapsing between skin penetration by cercariae and the first output of ova in the excreta. This time lapse is variable but the majority of authorities consider that it is about 12 or more weeks after skin penetration. Hence, recent opinion has favoured a decision on the therapeutic efficiency of drugs in an endemic area being reached 12 weeks after treatment. This appears a rational compromise since most preinfections and

drug failures would be manifest at this time and most reinfections immature. However, the data presented in Table 22 may lead to a revision of this opinion. In Table 22 are tabulated the times at which miracidia first appeared in the urine in patients classified as failures.

In patients completing a course of antimonial treatment, 15 out of 25 (60%) of failures appeared between 1 and 3 months after treatment, 6 out of 25 (24%) appeared between 3 and 6 months, 2 out of 25 (8%) between 6 and 9 months and 2 out of 25 (8%) between 9 months and 1 year.

Those failures who received incomplete courses presented a contrasting picture, 13 out of 15 (87%) becoming positive between 1 and 3 months after treatment and 2 out of 15 (13%) between 3 and 6 months. No further failures were detected after 6 months.

The importance of these findings is that between 80% and 90% of failures after antimony treatment will become manifest in the first 6 months, particularly in those who receive incomplete courses. A decision on comparative therapeutic efficiency made at 3 months, in this series, would have excluded a high proportion of positives occurring after this time and which were possibly treatment failures.

Relapse means therapeutic failure. There is no way of distinguishing, by examination of excreta, between relapse and a maturing preinfection or a reinfection. For comparative purposes it was decided to label all positive cases occurring from 1 to 6 months as treatment failures, realizing that an occasional preinfection might have occurred. The evidence suggested that by far the greatest proportion of treatment failures were relapses. Four failures in a total of 25 (16%), after a complete course, became manifest at 9 months or 1 year after previously being negative at monthly examinations. It was justifiable to regard these cases as true reinfections rather than as delayed treatment failures. It is recommended that, even in endemic areas, post-treatment examinations be continued for 6 months and assessments on drug efficiency be made from the data accumulating during this period, rather than assessment at an arbitrary point in time such as 3 months after treatment.

If our interpretation of positive cases of late onset as true reinfections is correct, then the role of reinfection seems to have been overemphasized in the past, at least in adults. Only 4 of a total of 40 failures (10%) became positive after the 6-month follow-up, a surprising finding in view of the high chances of re-exposure in the peasant population of this endemic area. The findings demonstrated that it is possible to conduct trials of antischistosomal drugs in endemic areas without the spectre of reinfection utterly confusing the results.

Importance of black eggs in follow-up urine examinations

The follow-up results (Tables 19, 20 and 21) showed that most patients passed black eggs in the urine. From a community viewpoint, such cases are harmless but in the individual there may be doubts whether true cure has been achieved. Despite the authoritative assertion of the WHO Expert Committee on Bilharziasis (1953) that " It is well known that dead eggs can be shed into urine or stool for months after cure ", some clinicians have adhered to the belief that only consistently negative excretal examinations after treatment constitute a cure, and have reserved their position on the significance of black eggs. In our experience one of the difficulties of rigid classification of follow-up material—e.g., no eggs, black eggs constant, black eggs intermittent—is the frequency of class interchange. Patients pass from one class to another by the occasional production of black eggs in the urine.

To clarify the significance of such class interchange a retrospective search of records was made to discover the eventual parasitological fate of patients who, after a complete treatment, were egg-free in early follow-up and those who passed black eggs, constantly or intermittently. The results are shown in Table 23.

Of 57 patients who, after a complete course of drug, passed black eggs during early follow-up, only 4 (7%) became failures at 6 months and a further 4 at 9 months or 1 year, giving a cumulative failure conversion rate of 14% during 1 year. The results suggested that the prognosis of patients passing black eggs in early follow-up, other factors being similar, did not differ greatly from the prognosis of the completely egg-free patients.

Recent experimental work in mice with mature *S. mansoni* infections, treated with conventionally curative courses of antimonials, or with a non-antimonial schistosomicide, 1-(5-nitro-2-thiazolyl)-2-imidazolidinone (niridazole, Ambilhar), showed at autopsy, conducted every 2 weeks until 6 months after treatment, that clusters of black eggs were always found in the wall of the small intestine and colon, although no adult schistosomes could be

TABLE 23

PROGNOSIS OF PATIENTS EXCRETING BLACK EGGS IN THE URINE AFTER ANTIMONIAL TREATMENT OF URINARY SCHISTOSOMIASIS

Classification of patient	Status at 1–3 months	Status at 6 months				Status at 12 months			
		C [a]	PC [b]	F [c]	Lost	C [a]	PC [b]	F [c]	Lost
Excreting no eggs (C) [a]	16	8	3	2	3	9	—	2	5
Excreting black eggs constantly (PC) [b]	20	2	13	2	3	2	5	6	7
Excreting black eggs intermittently (PC) [b]	37	16	11	2	8	14	5	2	16
Total	73	26	27	6	14	25	10	10	28

[a] Cure; no miracidia or ova in any specimen.
[b] Possible cure; no miracidia, dead eggs only found.
[c] Failure; miracidia isolated.

found (Lambert & Striebel, 1966). The relevance of this experimental study was obvious. It was concluded that the passage of small numbers of black eggs in the urine, after conventionally adequate treatment, in a patient who was not passing viable ova, was compatible with the concept of parasitological cure.

Summary

The results of immediate and late follow-up suggested that:

(1) The examination of excreta by an efficient method for 3 consecutive days during follow-up would detect all therapeutic failures. There is no need to examine over 5 days.

(2) 80%–90% of the failures would appear within 3 months. A small proportion, 1 or 2 in 10, may show a late relapse between 3 and 6 months after treatment.

(3) There was little therapeutic difference between AST intravenously and TWSb intramuscularly at equimetallic dosage. Both drugs, if given in adequate dosage, would cure the majority of cases of *S. haematobium* in adults in East Africa. TSAG was less effective at equivalent dosage but this was offset by the fact that tolerance was greatly superior.

(4) In adults, a course of 400 mg of metallic antimony gave a cure rate only slightly inferior to that after 500 mg. As the total antimony content of courses dropped below 400 mg the failure rate increased inversely.

(5) The evidence suggested that the cure rate was also inversely proportional to the pretreatment egg-output.

(6) The number of relapses, maturing preinfections or reinfections occurring at 6 months in an endemic area after treatment with a complete course or with a total dose greater than 400 mg of metallic antimony was not as great as had been expected. At 6 months the cumulative failure rate was known to be 24 out of 118 (22%), which rose at 1 year to 28 of 118 (24%).

(7) The failure rate in patients receiving a total dose of less than 400 mg of antimony, even those with low pretreatment egg-counts, was 38% at both 6 months and 1 year after treatment.

(8) The evidence suggested that a decision on the comparative value of schistosomicides should be made from the data acquired *during* a 6-month period of follow-up after treatment rather than at 3 months. Nearly all treatment failures (or maturing preinfections) will be manifest at 6 months. This is applicable to endemic areas or, with even greater force, to non-endemic areas.

(9) Follow-up results supported the concept that passage of black eggs after adequate antimonial treatment did not necessarily denote impending relapse. The presence of such eggs was compatible with parasitological cure.

(10) The long-term prognosis of patients passing black eggs or no eggs in early follow-up did not differ greatly.

URINARY CLEARANCE OF ANTIMONY [1]

Quantitative methods for the estimation of small amounts of antimony in biological materials include polarography (Page & Robinson, 1942; Goodwin & Page, 1943), chemical determinations (Maren, 1945, 1947) or detection of radioactive labelled compounds (Brady et al., 1945; Bartter et al., 1947; Abdallah & Saif, 1962). Recently, an accurate spectrographic procedure was devised (Kinser, Kupel & Keenan, personal communication, 1964).

Method

For routine clinical use, it was considered that a chemical method would be most satisfactory, and estimations of urinary antimony content were performed in selected patients by a modification of Maren's rhodamine B method (Maren, 1947) (see Annex for details).

During the first 10 days of treatment, 24-hour collections of urine were made. The urine was preserved with a small amount of chloroform and kept at a temperature of 4°C. Antimony estimations were performed in batches on 1-ml aliquots. Estimations were performed with the technician unaware of which antimonial the patients had received, until the series was completed.

The principle of the method involved digestion of the urine with oxidizing acids to produce pentavalent antimony. The digestate was than dissolved in concentrated hydrochloric acid, the antimony was extracted with isopropylether and combined with rhodamine B solution to form a coloured complex. Optical densities of unknowns were read on a spectrophotometer, and the amount of antimony present was determined by reference to the readings of simultaneously prepared standard solutions containing known amounts of antimony.

Results

The results of urinary antimony clearance estimations during therapeutic courses of TSAG, TWSb and AST are shown in Tables 24, 25, 26.

With equal doses of antimony in comparable patients with normal blood-urea levels, almost twice the amount of antimony given as TSAG was excreted in the urine as when given as AST. Antimony excretion during treatment with TWSb was only slightly higher than with AST. When constant daily dosage with TSAG was attained urinary antimony clearance was constant, some 38%–39% of the cumulative dose being cleared in 24 hours. With TWSb and AST, urinary antimony excretion was again fairly constant once daily dosages were constant, with the cumulative excretion showing a slight tendency to rise. In patients given AST some 16% of the cumulative dose was excreted at 4 days and 21% at 11 days. In patients given TWSb, 18% of the cumulative dose was excreted at 4 days and 27% at 11 days.

Urinary excretion is the major means by which antimony is eliminated from the body. These studies demonstrated that antimony retention during therapeutic courses at equimetallic dosage was greatest with AST, slightly less with TWSb and least with TSAG. In dealing with urinary clearance over 10–11 days on daily dosage with equal amounts of antimony in three different preparations, this work complements and extends previous observations.

A summary of trivalent antimony metabolism in man may be attempted. Parenteral administration of trivalent antimonials produced high but transient blood levels (Bartter et al., 1947). Red cell levels were higher than plasma levels but individual antimonials had different affinities for red cells (Lippincott et al., 1947; Gellhorn, Rose & Culbertson, 1947; Otto, Maren & Brown, 1947). Antimony rapidly left the plasma and appreciable tissue levels were found in the liver, the thyroid and the heart (Abdallah & Saif, 1962). There is probably a continuous shift of antimony from blood to tissues, which, combined with urinary excretion, complicates attempts to maintain high blood concentrations (WHO Scientific Group on Research in Bilharziasis (Chemotherapy), 1966). A total of 80% of excreted antimony is eliminated *via* the kidney and 20% *via* the faeces. Urinary clearance of antimony compounds is continuous, the rate varying with different preparations. It is suggested that urinary excretion of antimony after AST is similar whether the drug is given daily or on alternate days. Urinary excretion patterns after TWSb are broadly similar after daily or intermittent dosage. Due to slow continuous urinary excretion, a positive antimony balance always occurs during therapeutic courses, and final disposal of antimony after treatment may take some weeks (Lippincott et al., 1947; Abdallah & Saif, 1962).

Therapeutic implications

A review of previous work, and the results of present trials suggested that:

[1] This section was written by A. Davis and K. E. Broomfield (lately Chief Technician, WHO/MRC/Tanzania Bilharziasis Chemotherapy Centre).

(1) High renal clearance rates of antimony should minimize toxicity, and the results of treatment with TSAG confirmed this. TSAG is usually given over a shorter period than in the present trial, and the toxicity encountered by Rowland (1956) suggested that the excretion pattern was similar to that described here.

(2) Appreciable retention of antimony after AST or TWSb should give comparable toxicity values for equal dosages. This was confirmed.

(3) Since tissue retention occurs with all antimonials, increased dosage is more liable to provoke toxic reactions than to improve cure rates. In these trials, high cure rates were obtained with a metallic dose of 530 mg and it is unlikely that there would be therapeutic gain by increasing the metallic dosage beyond the 450-mg to 550-mg range.

(4) AST has the merits of low cost and a high cure rate but the long course of treatment results in large numbers of patients defaulting. Because of tissue retention of antimony, it is unlikely that shorter, effective regimes can be devised. It is doubtful whether this drug should be regarded as the standard schistosomicide for comparative purposes.

(5) The pattern of urinary excretion of antimony, although variable for different preparations, seems to conform to a common rule for an individual compound. Similar urinary antimony excretion patterns are seen whether TWSb is given daily or intermittently. Intermittent dosage generally diminishes side-effects and, since cure rates are high, a spaced dosage regime with this easily handled drug offers the best therapeutic compromise among the antimonial drugs in the treatment of *S. haematobium* infections.

(6) The risk of myocardial toxicity exists for some time following antimony treatment, because of tissue deposition and the slow excretion of the metal.

TABLE 24

URINARY EXCRETION OF ANTIMONY DURING TREATMENT [a] WITH INTRAVENOUS TRIVALENT SODIUM ANTIMONYLGLUCONATE

Day	Daily dose of metallic antimony (mg)	Cumulative dose of antimony (mg)	Urine volume per 24 hours (ml)	Antimony excreted in urine per 24 hours (mg)	Cumulative excretion of antimony in urine (mg)	Cumulative excretion of antimony as percentage of cumulative dose
	Means of 5 patients					
1	9.75	9.75	—	—	—	—
2	19.5	29.25	828	3.94	3.94	40.4
3	29.25	58.5	1 271	7.54	11.48	39.2
4	39.0	97.5	1 197	10.26	21.74	37.2
5	39.0	136.5	1 516	15.32	37.06	38.0
6	39.0	175.5	1 690	15.5	52.56	38.5
7	39.0	214.5	1 588	15.7	68.26	38.9
8	39.0	253.5	1 348	14.44	82.7	38.6
9	39.0	292.5	1 296	15.5	98.2	38.7
10	39.0	331.5	1 578	16.1	114.3	39.1
11	39.0	370.5	1 390	16.5	130.8	39.5
	Means of 2 patients					
12	39.0	409.5	815	12.9	143.7	38.8
13	39.0	448.5	950	14.6	158.3	38.7
14	39.0	487.5	1 050	16.5	174.8	39.0
15	39.0	526.5	830	14.2	189.0	38.8
16	—	526.5	1 055	16.6	205.6	39.1

[a] Means of patients weighing 52–59 kg; normal renal function.

TABLE 25

URINARY EXCRETION OF ANTIMONY DURING TREATMENT [a] WITH INTRAMUSCULAR ANTIMONY DIMERCAPTOSUCCINATE

Day	Daily dose of metallic antimony (mg)	Cumulative dose of antimony (mg)	Urine volume per 24 hours (ml)	Antimony excreted in urine per 24 hours (mg)	Cumulative excretion of antimony in urine (mg)	Cumulative excretion of antimony as percentage of cumulative dose
	Means of 4 patients					
1	10	10	—	—	—	—
2	20	30	1 152	1.4	1.4	10.4
3	30	60	1 141	4.0	5.4	18.0
4	40	100	1 139	5.2	10.6	17.7
5	40	140	1 390	9.0	19.6	19.6
6	40	180	1 528	11.4	31.0	22.1
7	40	220	1 295	10.0	41.0	22.8
8	40	260	1 083	13.4	54.4	24.7
9	40	300	1 243	13.1	67.5	26.0
10	40	340	1 153	13.0	80.5	26.8
	Means of 3 patients					
11	—	340	1 047	12.0	92.5	27.2

[a] Means of patients weighing 56–73 kg; normal renal function.

TABLE 26

URINARY EXCRETION OF ANTIMONY DURING TREATMENT [a] WITH INTRAVENOUS ANTIMONY SODIUM TARTRATE

Day	Daily dose of metallic antimony (mg)	Cumulative dose of antimony (mg)	Urine volume per 24 hours (ml)	Antimony excreted in urine per 24 hours (mg)	Cumulative excretion of antimony in urine (mg)	Cumulative excretion of antimony as percentage of cumulative dose
	Means of 6 patients					
1	9.4	9.4	—	—	—	—
2	20.0	29.4	1 026	1.0	1.0	10.6
3	29.5	58.9	1 366	3.1	4.1	13.9
4	40.1	99.0	1 217	5.4	9.5	16.1
5	40.1	139.1	1 397	7.5	17.0	17.2
6	40.1	179.2	1 497	7.3	24.3	17.5
7	40.1	219.3	1 407	9.7	34.0	19.0
8	40.1	259.4	1 040	9.6	43.6	19.9
9	40.1	299.5	1 242	9.4	53.0	20.4
10	40.1	339.6	1 093	8.9	61.9	20.7
11	40.1	379.7	882	11.0	72.9	21.5

[a] Means of patients weighing 50–66 kg; normal renal function.

GENERAL DISCUSSION AND CONCLUSIONS

Technique of clinical trials

In comparative clinical trials the formation of strictly comparable groups of patients for treatment and for control is essential, if valid deductions of drug effect are to be made from data collected. Smart (1963) summarized the position thus " The clinical experimenter is therefore normally forced to accept the fact that there will be substantial variations from one patient to another in respect of the characteristics which are relevant to his problem; and he must therefore arrange his trial in such a way as to ensure that, as far as possible, his results are not biased as a result of these differences ". There are two ways of approaching this problem. The first is by careful matching of patients or groups and the allocation of one of each pair or one of each group to different treatments. Matching requires admission to hospital and investigation. In this trial the multiplicity of parasitic conditions encoutered made the task of exact matching impossible, and the second method of ensuring freedom from bias, by random admission to hospital and random allocation to treatment groups, was adopted. Given sufficient patients from a common host population the laws of chance operate, and tend to ensure an equal distribution of pathological material among the treatment groups. The sample then reflects a broad spectrum of the disease and conclusions from the trial can be applied to all adults in the area.

The comparison of egg-counts performed during primary screening, and those later performed during timed collection periods in hospital, showed that external controls for assessment of drug action were not absolutely necessary however desirable they might be scientifically. Spaced egg-counts suggested that secular fluctuations in egg-output, in chronic disease in an endemic area, would be overwhelmingly unlikely to confuse therapeutic assessment.

These trials were termed " comparative " since double-blind procedures were impracticable under the prevailing circumstances. Although a blind procedure could be adopted in comparing two drugs given intravenously, the presence of an intramuscularly administered drug was a complicating factor. For statistical validity, the reference and the control groups should be handled in the same way.

It has been stated that comparative evaluations are significant only when the optimum dosage had been previously established (Friedheim, unpublished communication to WHO, 1964). But it was precisely because the optimum dose of antimony had never been determined that a fixed dosage scheme was adopted. In the present state of knowledge, it may be questioned whether any dose of antimony can be defined precisely as optimum for all strains of the parasite and for all parts of the world. The dosages selected were a practical compromise.

The advantage of a fixed-dose trial is that generalization of the results is more secure than after variable dose regimes (Mainland, 1963). It may be more desirable to imitate the conditions of ordinary clinical practice by allowing the observer to regulate the dose according to his opinion of the patient's tolerance. However, 10 different physicians may well have 10 different opinions on the patient's tolerance which would merely confuse the issue. What is important is that strict adherence to a predetermined dose regime is unjustifiable in a clinical situation involving a toxic drug. In these trials it was hoped to treat all patients with a fixed total dose but toxicity rendered this impossible. One physician was responsible for all assessments and decisions to stop treatment; this reduced observer variation, but individual bias, conscious or unconscious, was inevitable. Clinical trials should never be the responsibility of a lone physician. A good case can be made for multicentre trials guided from a comprehensive protocol.

Host population

The over-all picture of the human host population in this area is similar to that of rural Africans over wide areas of the continent and Gelfand (1961) and others have pointed this out repeatedly. Hepatosplenomegaly was present in some 35% of our patients and multiple parasites were harboured by 75%–80% the most common being hookworm and *Wuchereria bancrofti*. No attempt was made to deal with infections other than malarial parasitaemia.

The high incidence of macroscopic radiological abnormalities agreed with the findings of previous authors. Honey & Gelfand (1960) have shown by functional studies that such lesions may carry a serious prognosis. The association between radiological abnormality and high urinary egg-output in adults agreed with the conclusions of Forsyth & Macdonald (1965) in children. This finding in a group of young adults, 40%–50% of whom had received some treatment, emphasized that inadequate treatment does nothing to diminish the gravity of the prognosis and efforts to eradicate the infection must be made in childhood.

Forsyth & Macdonald (1965) showed that ureteric and bladder damage can occur in a primary infection and that there was no lower limit of egg-output at which abnormal X-ray appearances could be seen. Thus, it was scarcely surprising that 34% of the pyelograms in this sample were abnormal. The extensive studies of the Ross Institute team in the last few years (for instance, those of Forsyth & Bradley, 1964) have demonstrated the high incidence of radiological abnormality in apparently healthy schoolchildren in an endemic area, and there has been much speculation for and against the possibility of early death. Until more is known of the importance of the pathology of urinary schistosomiasis in different types of populations, many public health authorities will continue to regard the disease with scepticism and will continue to give a low priority to control measures.

Chemotherapeutic considerations

Equivalent amounts of antimony, given by means of different preparations to comparable host groups infected with the same strain of parasite, did not have the same therapeutic effect. Excellent cure rates were obtained with intravenous AST and intramuscular TWSb, but toxicity proved a serious problem. Tolerance to intravenous TSAG was greatly superior although cure rates were slightly inferior. While a broad association between therapeutic efficiency and tissue retention of antimony can be inferred, further speculation would be profitless in the absence of more detailed knowledge of the pharmacology of antimony.

Each of the three drugs tested had merits according to circumstances; AST was cheap and gave excellent cure rates; TWSb gave excellent cure rates and was easily administered; TSAG had superior tolerance and gave good cure rates. Two of the drugs, TWSb and TSAG, are commonly given over shorter periods—a major advantage—and courses of treatment can be completed in times ranging from 4 to 8 days. There is no completely satisfactory method of shortening the time of administration of antimony sodium tartrate. An over-all recommendation applicable to widely varying circumstances has been difficult, if not impossible, to reach. The literature shows the value of intermittently spaced injections of TWSb. Courses of treatment, in which the drug is given on alternate days, twice, three times, or even once weekly, commend themselves for the treatment of large numbers of people. Cure rates are high and tolerance is reputedly superior to that encountered in the present trial. TWSb should not be used in daily dosage and the exact details of spaced dosages must be a matter for local decision. For patients who have defaulted from previous treatment with other antimonials on grounds of toxicity, TSAG would prove a satisfactory alternative in *S. haematobium* infections. The course of treatment can be conveniently shortened but the necessity for intravenous injections is a disadvantage.

None of these drugs is suitable for mass treatment. All are toxic and should be given under trained supervision. The management of specific groups of infected individuals, e.g., schoolchildren, armed services, labour forces, would be possible only if medical supervision could be guaranteed. Mass chemotherapy among rural populations, who constitute the major reservoir of the disease, remains unlikely with the antimonials even if widespread coverage from rural health centres was available. Until the advent of some effective, though much less toxic, schistosomicide, the control of the disease by mass chemotherapy, a form of attack which had great theoretical possibilities (Macdonald, 1965), is likely to remain a practical impossibility.

Suggestions for the conduct of future research

In the protocol given in Annex 1 to the report of the WHO Scientific Group on Research in Bilharziasis (Chemotherapy) (1966), it was suggested that much of the basic work on schistosomicidal chemotherapy should be carried out in centres located in endemic and non-endemic areas. The ideal would be the creation of new centres, and failing this, centres would be formed in established institutes, their autonomy being assured.

Experience at this prototype centre suggests that, for chemotherapeutic assessment of drugs, serious consideration be given to multicentre trials based on existing institutions. Such trials would avoid the expense of building new centres, which, as the present project has shown, will have difficulty in recruiting trained personnel. Much of the work of a centre is still concerned with conditions in the field and unless a projected new centre can be adequately staffed from its inception, then its diverse functions as envisaged in the protocol cannot be undertaken, and the most that can be expected is small-scale work directed towards the solution of a particular problem.

Multicentre trials based on existing facilities are an accepted feature of current medical research.

The Medical Research Council of Great Britain, among others, have demonstrated their undoubted success in such diverse conditions as pulmonary tuberculosis, the rheumatic diseases and depressive illness. There are research institutes in most of the major endemic areas. The essentials for success would be competent full-time investigators already in the field who would agree to participate. They would form a central committee to formulate an agreed protocol on methods of conducting and assessing trials. The crux of the matter would be agreement on standardized methods of examination. Much of the information in the WHO protocol would be utilized directly and combined with previous recommendations of WHO Scientific Groups and Expert Committees. A competent and tactful co-ordinator at a central base would be a necessity.

It is unlikely that any one "standard" antimonial drug can be used as an absolute measure of therapeutic efficiency. In the protocol referred to above it is suggested that a reference drug is essential. AST is not the most suitable preparation; intramuscular TWSb, in spaced administration, is more suitable. Multicentre trials, using a common protocol, would give a clearer picture than would the results from any one particular centre, or from individual workers. Under the present system of field research comparative trials will still play an important part in the assessment of schistosomicides.

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RÉSUMÉ

Il n'est pas aisé de comparer valablement l'activité schistosomicide des antimoniaux en raison de la diversité des préparations, des dosages, des méthodes thérapeutiques et des critères de guérison utilisés au cours des évaluations. Aussi l'Organisation mondiale de la Santé a-t-elle publié un protocole détaillé qui définit entre autres les modalités des essais. D'autre part, à la suite d'un accord entre le Gouvernement de la République-Unie de Tanzanie, l'OMS et le British Medical Research Council, un Centre de Chimiothérapie de la Bilharziose a été établi à Tanga, Tanzanie. Des recherches sur les schistosomicides y sont menées conformément aux principes formulés par les groupes scientifiques de l'OMS.

Au cours de l'étude dont les résultats sont rapportés dans le présent article, trois antimoniaux ont été soumis à des essais d'efficacité. Trois groupes de malades africains adultes atteints de schistosomiase urinaire (*Schistosoma haematobium*) ont été constitués. Le groupe A (60 malades) a reçu du gluconate d'antimonyle et de sodium (TSAG) par voie intraveineuse; le groupe B (50 malades) a été traité par injections intramusculaires de dimercaptosuccinate d'antimoine (TWSb); le groupe C (50 cas) a reçu du tartrate d'antimoine et de sodium (AST) par voie intraveineuse. Pour éviter les variations dues aux dosages, les schémas de traitement ont été adaptés de manière à fournir à chaque sujet une dose quotidienne équivalente d'antimoine, la dose totale, à la fin du traitement de 15 jours, étant d'environ 530 mg d'antimoine métallique. Les examens cliniques effectués avant le traitement n'ont mis en évidence aucune différence significative entre les trois groupes. Ils ont porté essentiellement sur l'intensité de l'élimination d'œufs dans l'urine, le poids corporel, la présence d'une hépato- ou splénomégalie et l'existence d'autres parasitoses. L'ankylostomiase, le paludisme et la filariose à *Wuchereria bancrofti* étaient fréquentes, et une urographie pratiquée sur un échantillon de 100 malades a montré des anomalies radiologiques dans un tiers des cas.

Au cours du traitement, on a procédé quotidiennement à la mesure de l'élimination des œufs par l'étude de l'éclosion et le comptage des œufs morts contenus dans un échantillon de l'urine recueillie entre 10 et 14 heures. Des examens de contrôle ont montré qu'il n'y avait pas lieu de tenir compte des fluctuations occasionnelles de l'excrétion des œufs au moment d'évaluer l'efficacité d'un médicament après le traitement.

La tolérance aux trois médicaments a été très différente. C'est ainsi qu'en raison de la gravité des effets secondaires, le traitement a dû être interrompu chez 3% des patients traités par le TSAG, 52% des patients traités par le TWSb et 70% des malades recevant de l'AST.

Des examens d'urine ont été pratiqués, pendant 3 jours consécutifs, 1, 2, 3, 6, 9 et 12 mois après la fin du traitement. On n'a noté que peu de différence entre l'activité thérapeutique du TWSb et celle de l'AST, ces deux produits, à doses suffisantes, guérissant les malades qui avaient reçu un traitement complet. Le TSAG s'est montré moins efficace, mais a été beaucoup mieux toléré. Les taux de guérison ont été proportionnels à la dose totale et inversement proportionnels à l'intensité de l'élimination des œufs avant le traitement. Dans 80 à 90% des cas, les échecs ont été manifestes dans les 3 mois suivant le traitement. L'évaluation de l'efficacité des schistosomicides devrait être basée sur les taux cumulatifs d'échecs durant les 6 mois après le traitement plutôt que sur l'appréciation des résultats à une date fixe. Le taux cumulatif d'échecs chez les patients recevant une dose totale de plus de 400 mg d'antimoine métallique a été de 24% après un an. La persistance de l'élimination d'œufs morts après le traitement schistosomicide n'est pas incompatible avec la guérison parasitologique.

L'étude de l'excrétion urinaire de l'antimoine montre que la rétention du métal dans l'organisme est maximale après administration de AST, légèrement inférieure après traitement par le TWSb et moins élevée encore si l'on utilise le TSAG.

Bien que chacun des composés soumis à cet essai ait fait preuve de certains mérites, on ne peut envisager de les utiliser dans le cadre d'un traitement de masse, à cause de leur toxicité et de la nécessité de surveiller le traitement de près.

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Annex

ESTIMATION OF ANTIMONY BY THE RHODAMINE B METHOD

All chemicals used were analytical reagent grade. A known volume of urine was placed in a micro-Kjeldahl flask after thorough mixing of the original specimen. Exactly 1 ml of concentrated perchloric acid (60%) and 2 drops of capryl alcohol were added. After thorough mixing, the flasks were placed in a digestion stand and heated to 200°C. Solutions turned pale yellow, dark brown, then pale yellow again. The flasks prevented loss from spitting which may occur if boiling-tubes are used. Copious white fumes of perchloric acid were given off, then much less copious white fumes of sulfuric acid, and oily drops were deposited on the sides of the flasks. Digestion was complete when effervescence ceased and the flasks were then removed from the heater. When cool enough to avoid cracking, the flasks were placed in crushed ice, 5 ml of concentrated hydrochloric acid were added and the contents of the flasks were transferred to a separating funnel. The flasks were washed with 5 ml of concentrated hydrochloric acid added to the separating funnel. After 15 ml of isopropylether had been added, the funnels were shaken for 30 seconds, then 5 ml of distilled water was added and mixed. The funnels were left in the rack for 10 min, then shaken for 30 seconds, and the bottom layer was run off and discarded. A 10-ml volume of rhodamine B solution (0.02% in 0.5N HCl) was added to the funnel which was shaken for 30 seconds and the bottom layer was discarded. The top layer was transferred to a test-tube and centrifuged, when necessary, to remove turbidity.

Optical densities were read in a Coleman 6C junior spectrophotometer with a 35-mμ band width, in matched round cuvettes of 19-mm diameter against a reagent blank (1 ml of distilled water taken through full procedure). Extraction with 15 ml of isopropylether maintained readings within the extinction range 0.2–0.8 and there was a marked absorption peak at 550 μm, at which wavelength all readings were made.

Duplicate unknowns, duplicate reagent blanks and duplicate standard solutions of antimony (two strengths) were included in each batch of estimations. The mean of the reagent blank readings was subtracted from the mean optical density of the unknowns and from the means of the two different strengths of standard antimony solutions (5 μg/ml and 15 μg/ml) and the antimony concentration of unknowns was calculated.